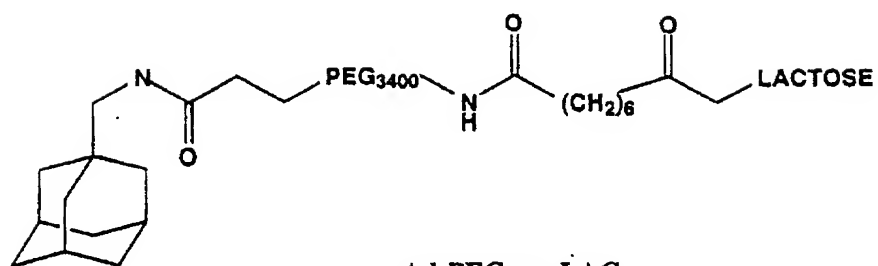
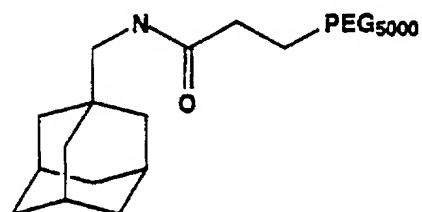


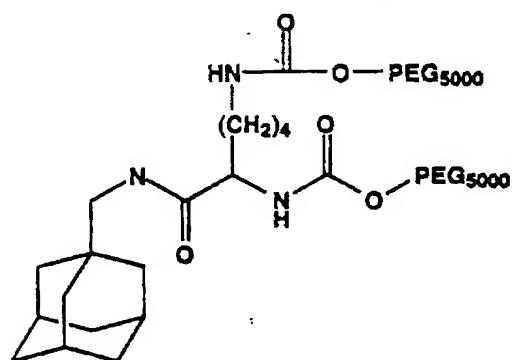
Ad-PEG<sub>3400</sub>



Ad-PEG<sub>3400</sub>-LAC



Ad-PEG<sub>5000</sub>



Ad-(PEG<sub>5000</sub>)<sub>2</sub>

Figure 1 Structures of Various Adamantane-PEG molecules.

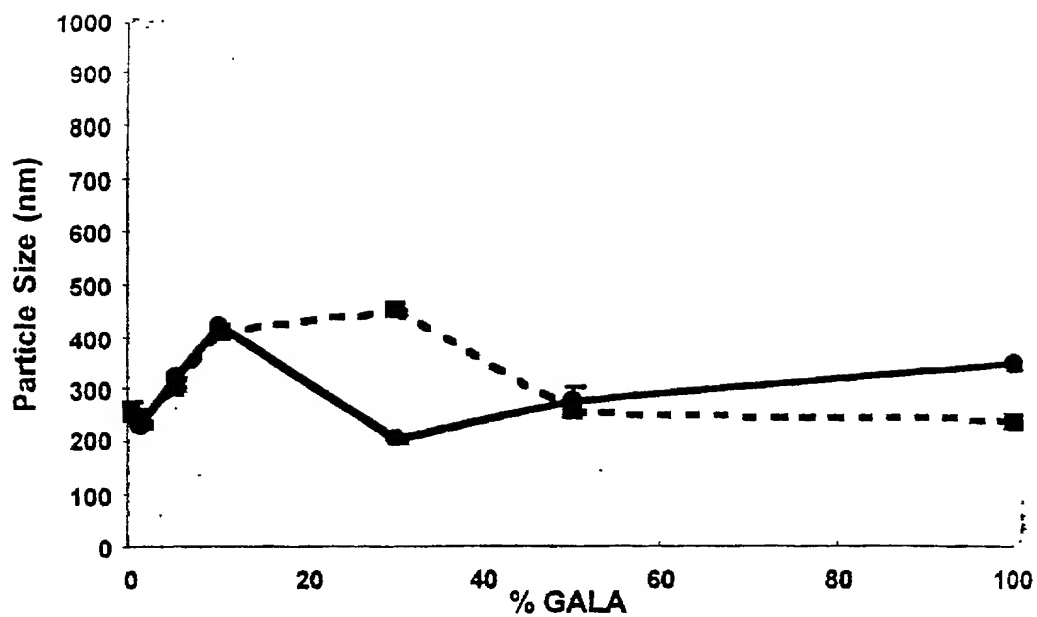


Figure 2. Hydrodynamic diameter of GALA (dashed line) and GALA-Ad (solid line)-modified polyplexes.

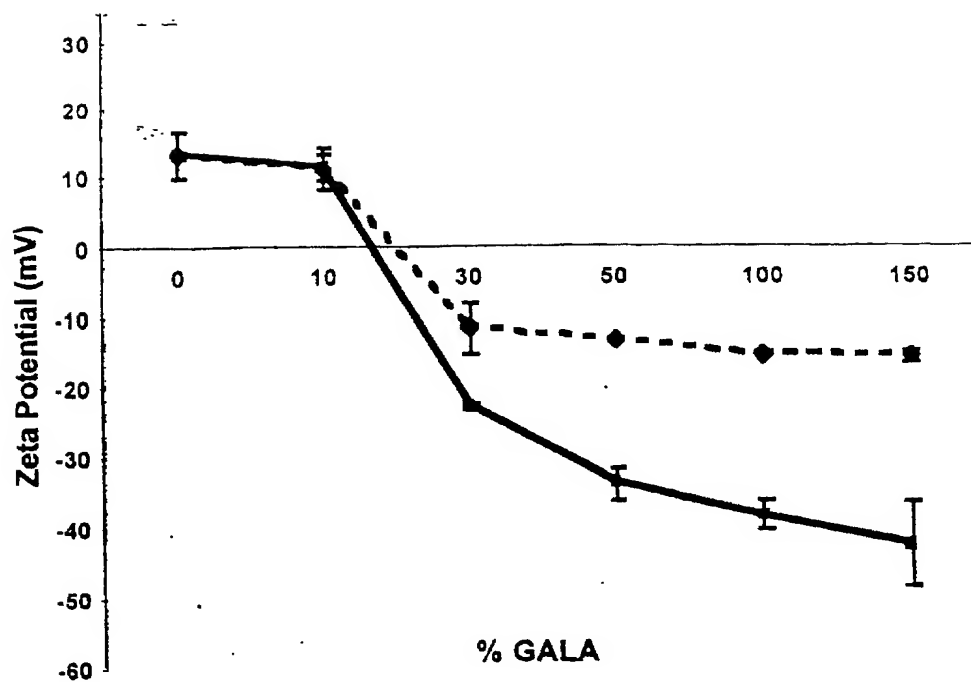


Figure 3. Zeta potential of GALA (dashed line) and GALA-Ad (solid line)-modified polyplexes.

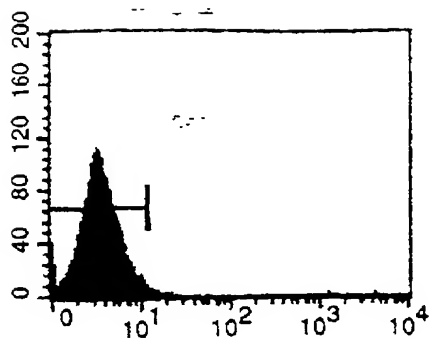


Fig 4a. Untransfected BHK-21 cells

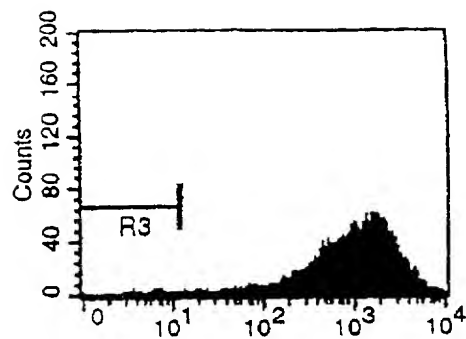


Fig 4b. BHK-21 cells transfected with  $\beta$ CDP6/FITC-Oligo at 5+/-

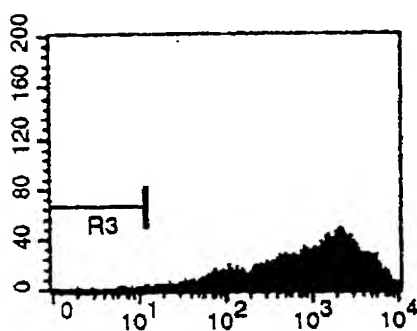


Fig 4c. BHK-21 cells transfected with  $\beta$ CDP6/FITC-Oligo/50% GALA

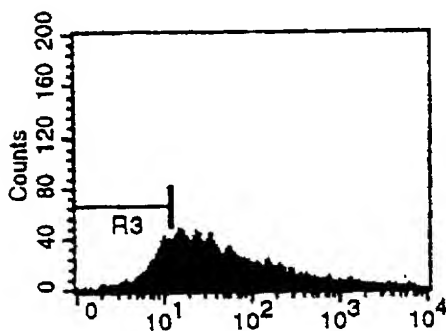


Fig 4d. BHK-21 cells transfected with  $\beta$ CDP6/FITC-Oligo/50% GALA-Ad

Fig 4. Uptake of GALA-Ad and GALA modified polyplexes by BHK-21 cells.

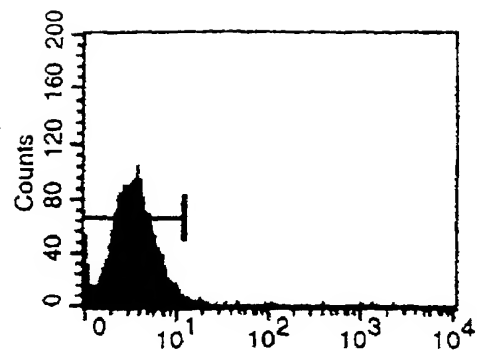


Figure 5a. Untransfected HUH-7 cells

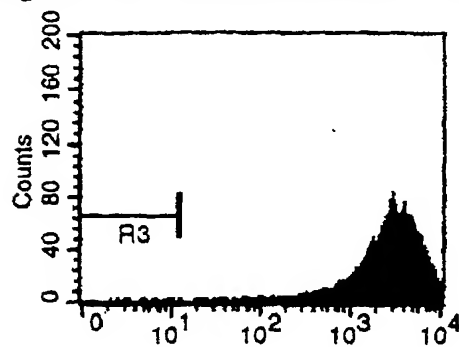


Figure 5b. HUH-7 transfected with  $\beta$ CDP6/FITC-Oligo at 5+/-

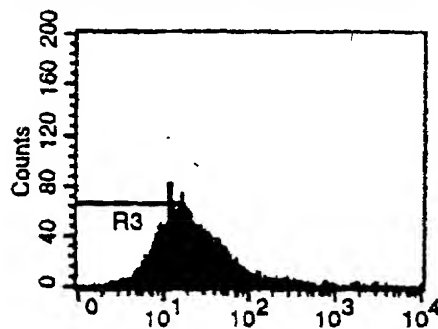
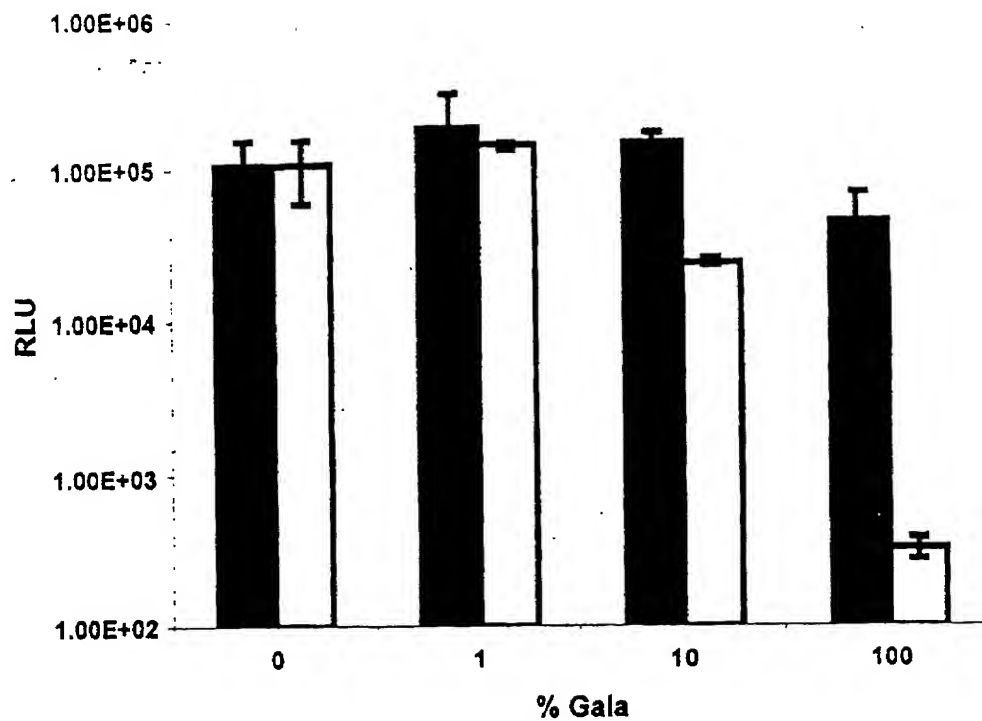


Fig 5c. HUH-7 transfected with  $\beta$ CDP6/FITC-Oligo/ 50% GALA-Ad

Fig 5. Uptake of GALA-Ad and GALA modified polyplexes by HUH-7 cells



**Fig 6.** Luciferase transfection of BHK-21 cells with  $\beta$ CDP-based polyplexes modified with GALA (shaded bars) and GALA-Ad (white bars).

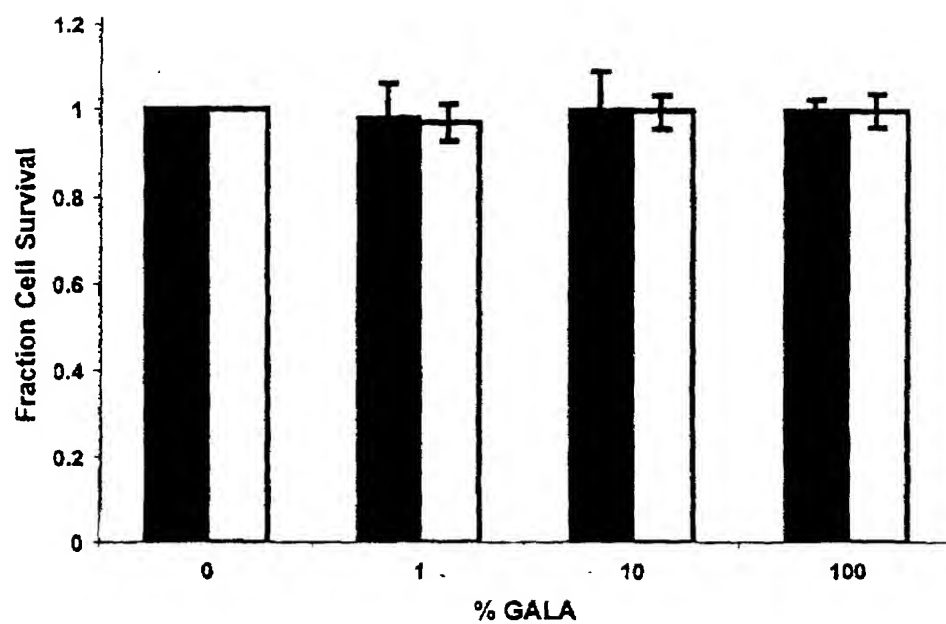


Fig 7. Toxicity of GALA and GALA-Ad modified polyplexes to BHK-21 cells.

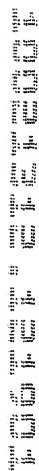


Figure 8: Scheme for post-DNA-complexation pegylation by grafting.



| Polyplex          | PEG        | Stage 1 (nm) | Stage 2 (nm) | Stage 3 (nm) |
|-------------------|------------|--------------|--------------|--------------|
| PEI 3+/-          | 10:1       | 58           | 65           | 115          |
| PEI 6+/-          | 10:1       | 55           | 60           | 78           |
| $\beta$ CDP6 5+/- | 100%       | 70           | 67.4         | 303          |
| $\beta$ CDP6 5+/- | 150%       | 70           | X*           | N/A          |
| $\beta$ CDP6 5+/- | 200%       | 70           | X*           | N/A          |
| $\beta$ CDP6 5+/- | 100% PEG** | 67           | 81           | 700          |

\*Poor correlation function; no size measurements possible.

\*\*PEG<sub>5000</sub> added instead of PEG<sub>5000</sub>-SPA

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**Figure 9: Particle sizes of PEI and 12 ( $\beta$ CDP6) polyplexes during post-DNA-complexation pegylation of grafting.**

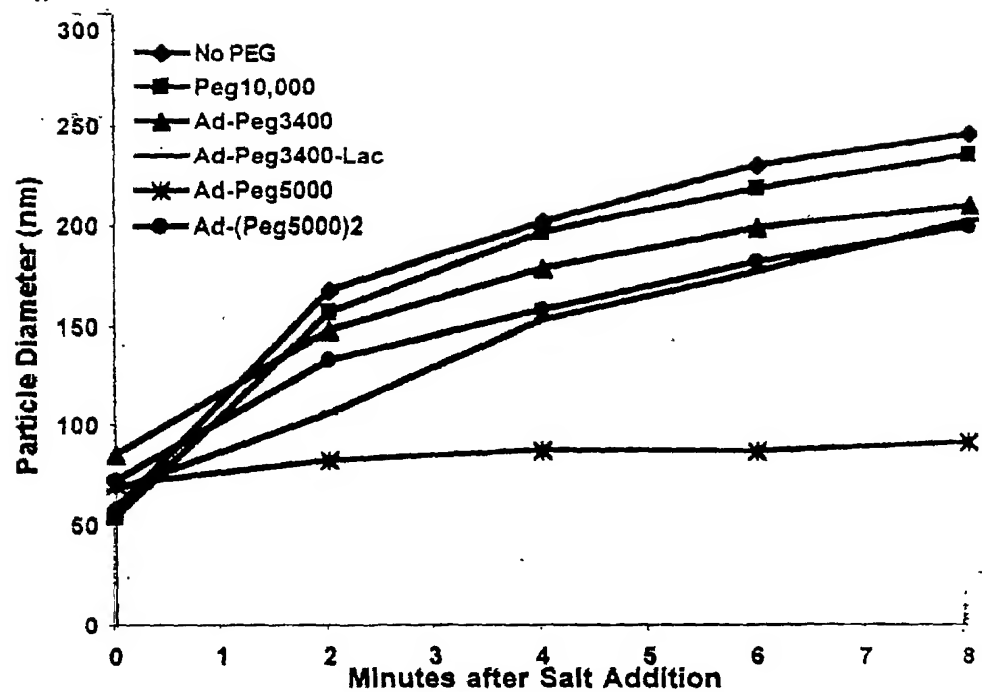


Fig 10 Salt stabilization of polyplexes by pegylation.

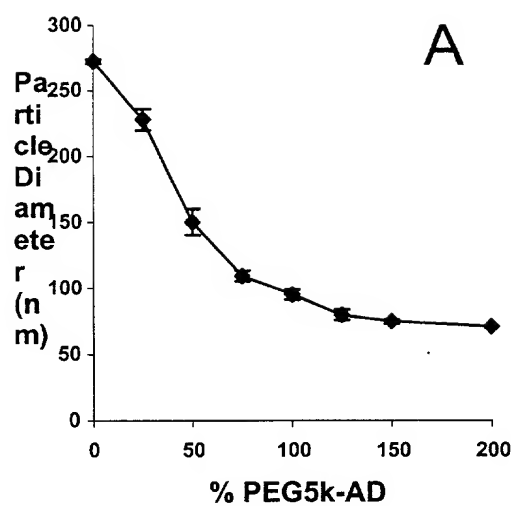


Figure 10A: Stabilization of polyplexes by pegylation.

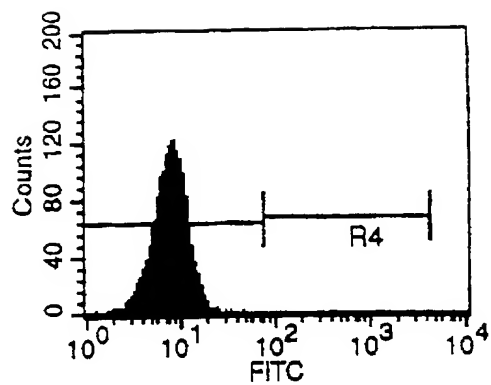


Fig 11a Untransfected HUH-7

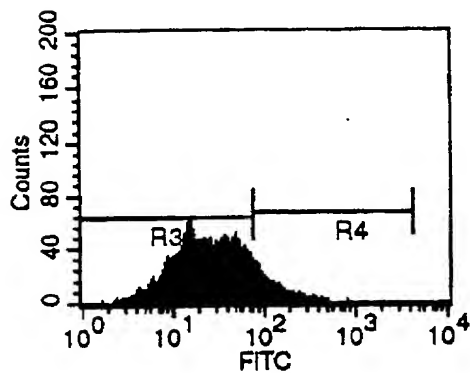


Fig 11b HUH-7 transfected with  $\beta$ CDP6/Oligo + free PEG<sub>3400</sub>-FITC

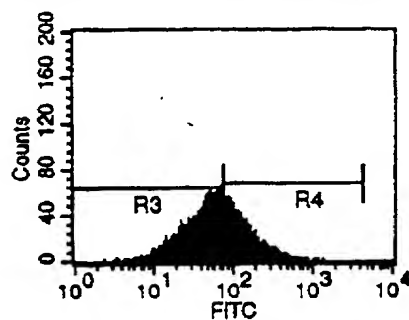
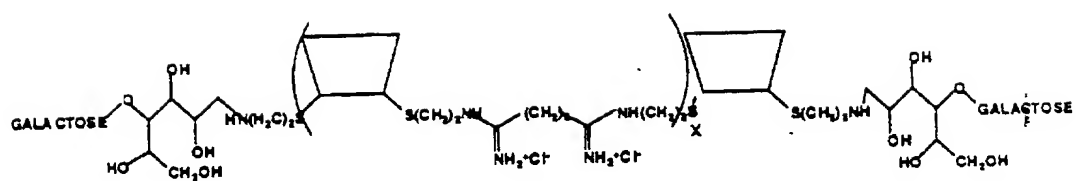


Fig 11c HUH-7 transfected with  $\beta$ CDP6/Oligo/Ad-PEG<sub>3400</sub>-FITC

Fig 11 Co-delivery of  $\beta$ CDP6 polyplexes with PEG<sub>3400</sub>-FITC.



**Fig 12 Structure of Lactose-CDP6.**

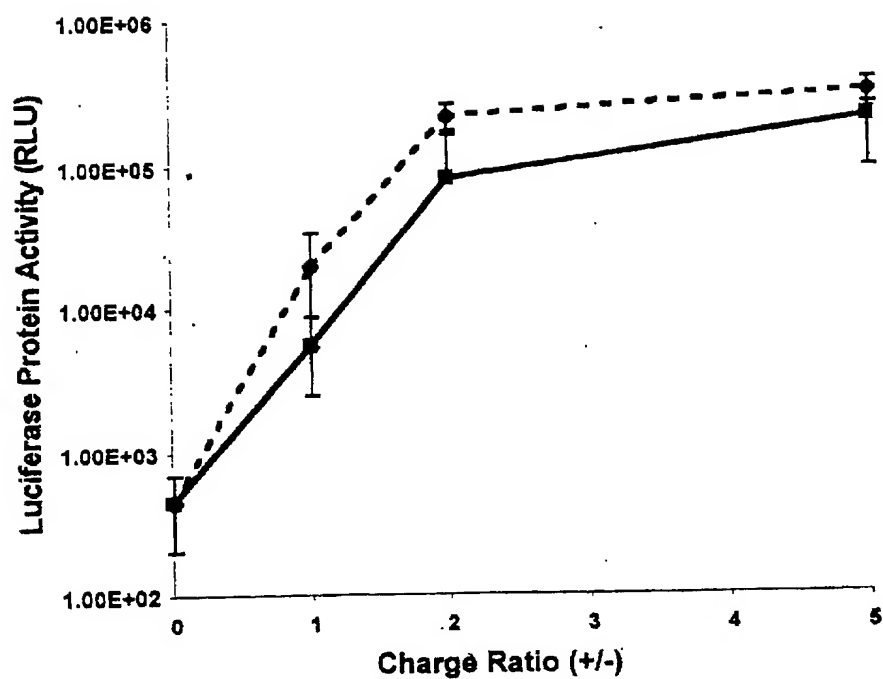
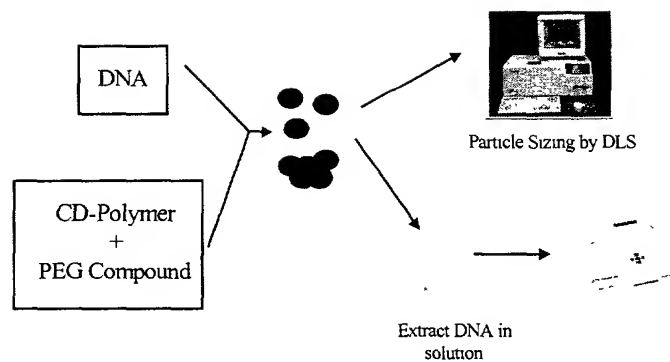


Fig 13 Transfection of  $\beta$ CDP (dashed line) and Lac-CDP6 (solid line) polyplexes to HUH-7 cells.



10 Figure 14. Schematic of Experimental Protocol, Example 47

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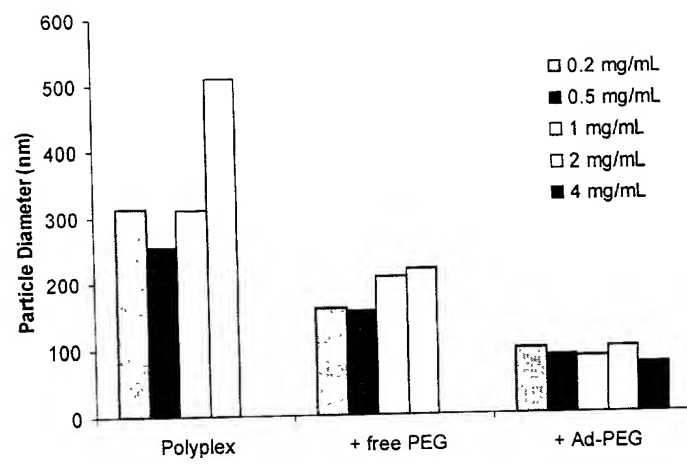


Figure 15. Particle Diameter



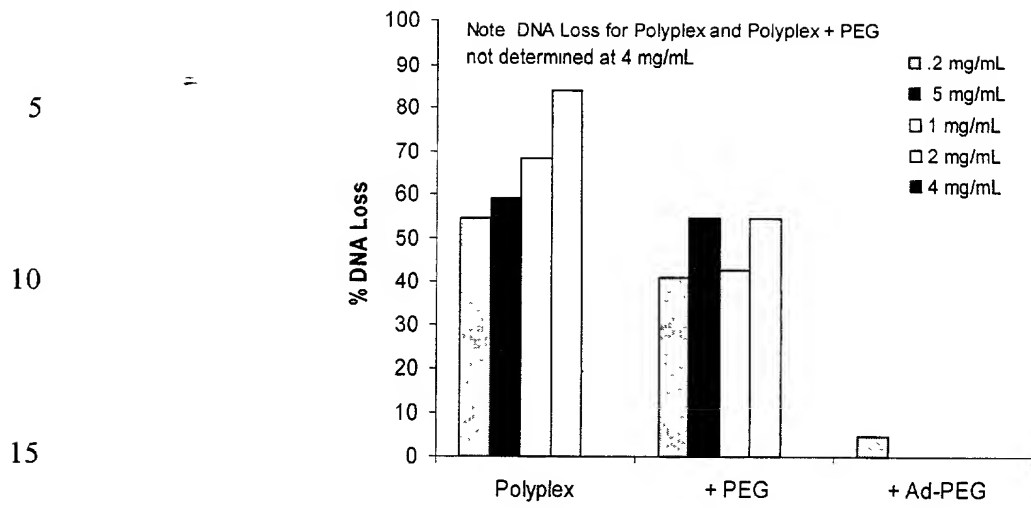
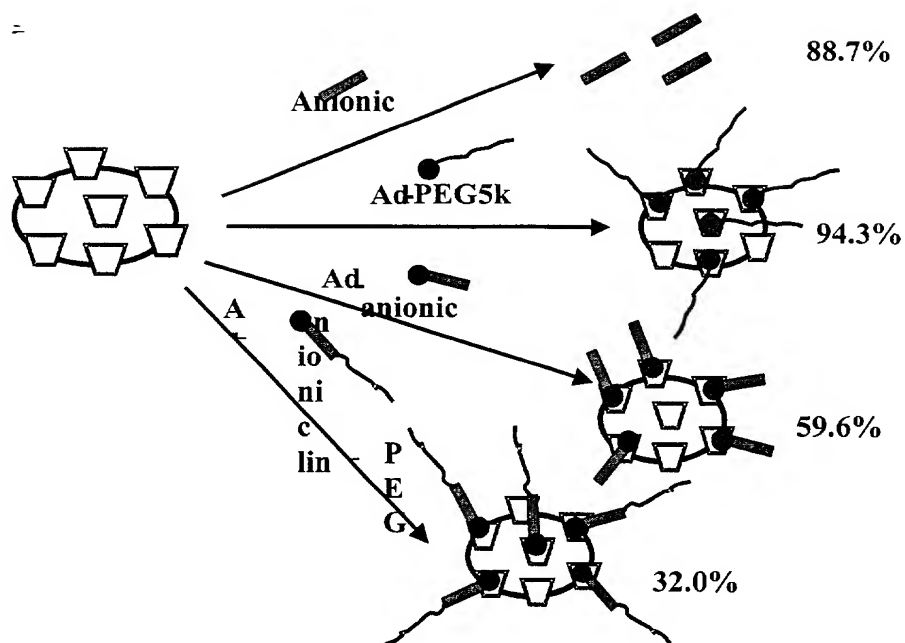
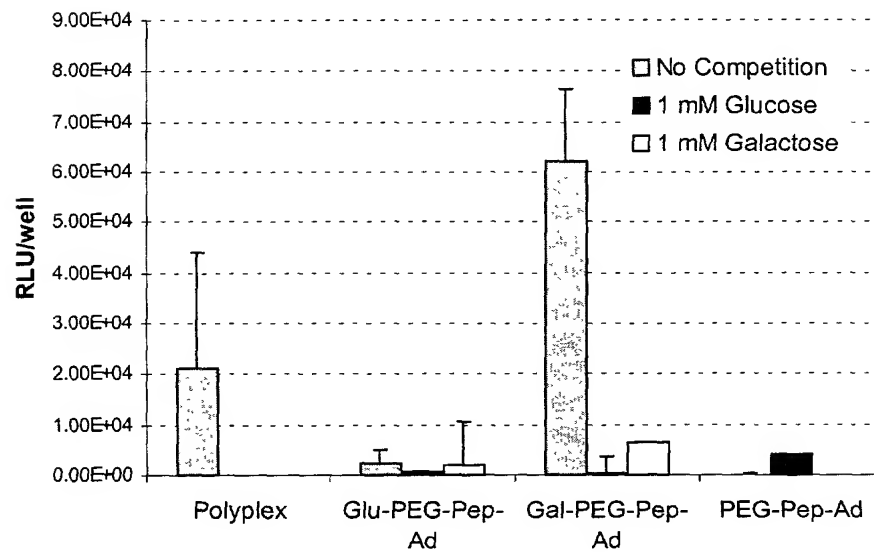


Figure 16. DNA Loss Due to Complex Precipitation



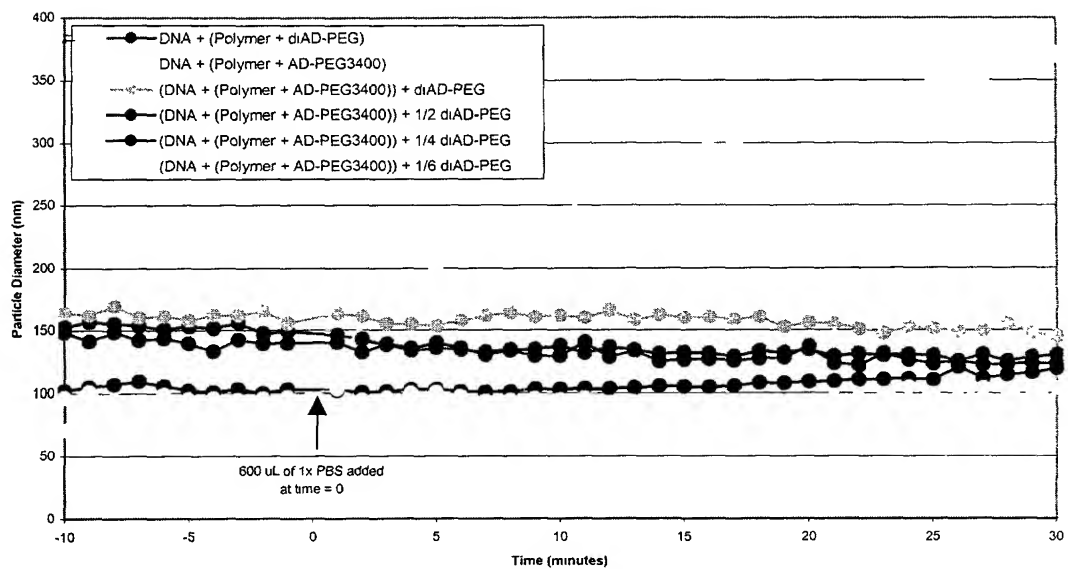
5 Figure 17 Inclusion Complexes to Modify 12/DNA Composite

**Transfection of Modified Polyplexes to HepG2 cells  
(50% PEG)**



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Figure 18



5 Figure 19 Competitive Displacement Experiments

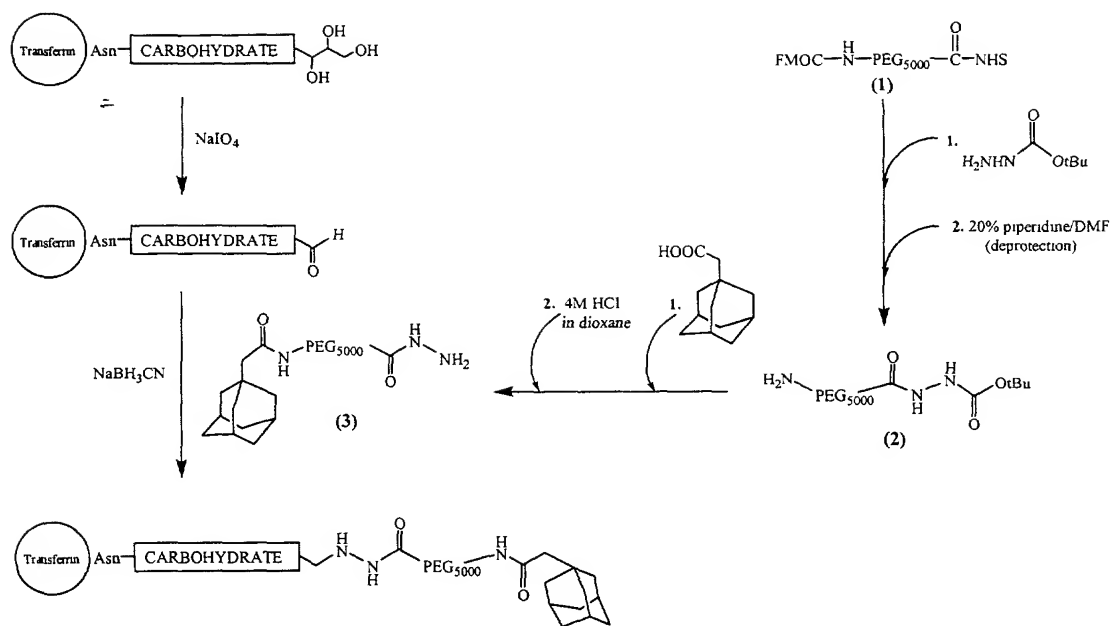


Figure 20 Synthesis of Adamantane-PEG-Transferrin (Ad-PEG-Tf)

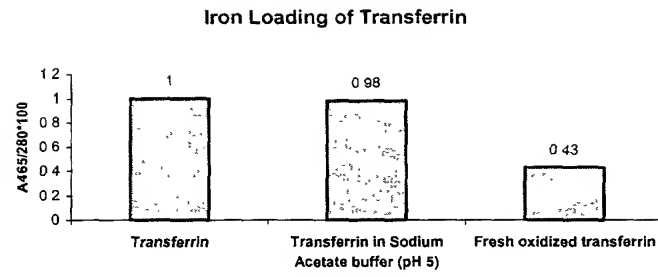


Figure 21

Figure 21 shows the iron loading of transferrin. The y-axis represents the ratio of absorbance at 465 nm to 280 nm, multiplied by 100 (A465/280\*100). The x-axis shows three samples: Transferrin, Transferrin in Sodium Acetate buffer (pH 5), and Fresh oxidized transferrin. The values for each sample are 1, 0.98, and 0.43, respectively.

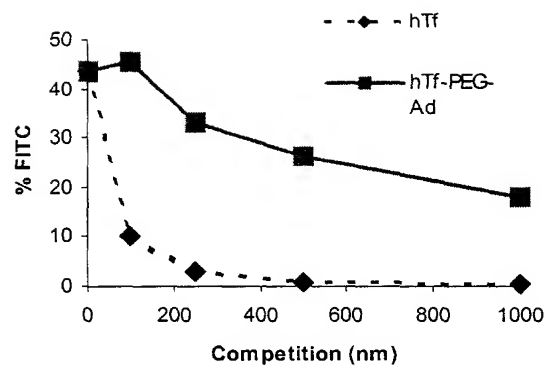
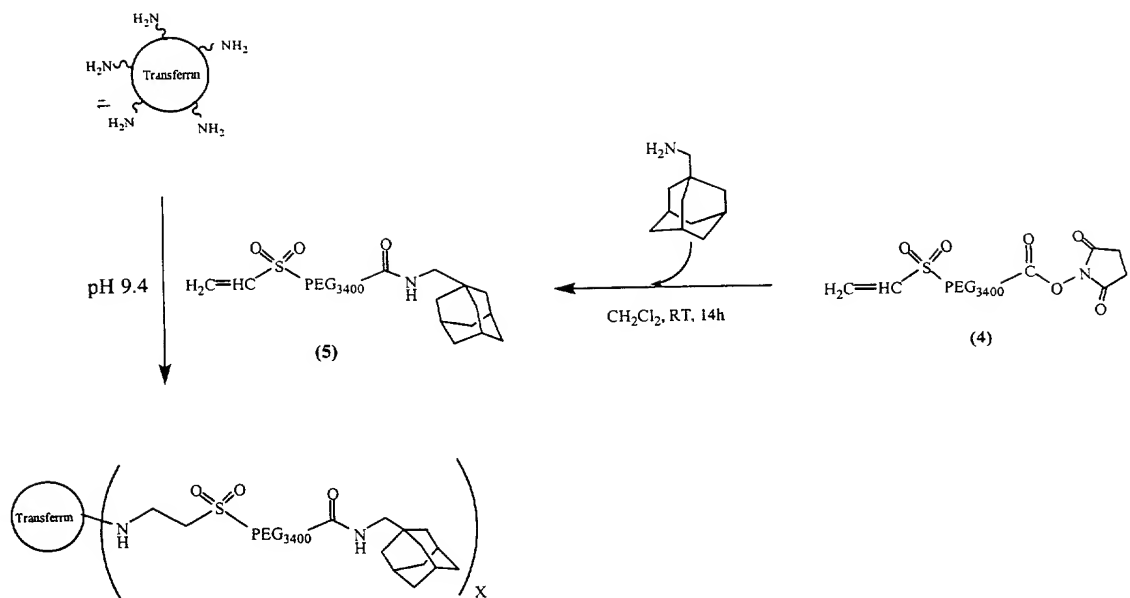
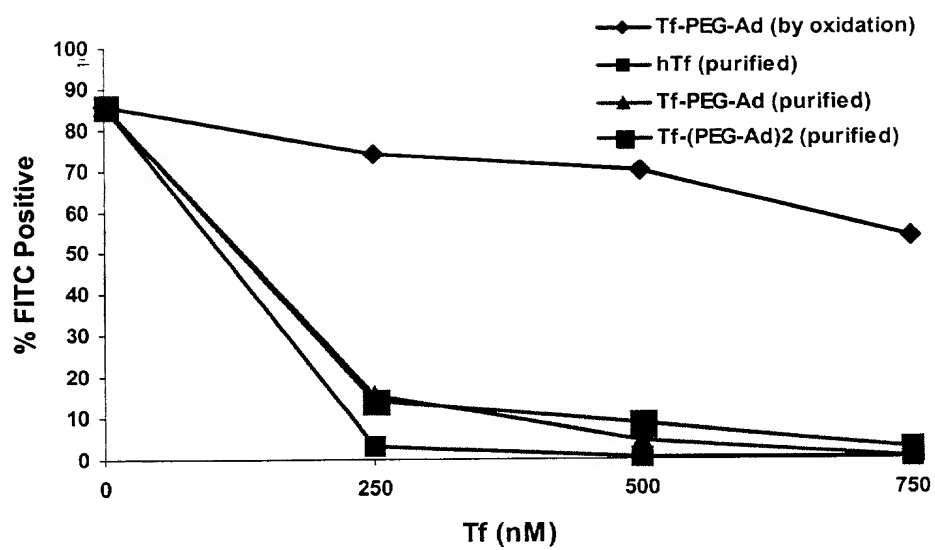


Figure 22 Binding Affinity Transferrin-PEG-Ad



5 Figure 23 Transferrin coupling via Lysine groups





5 Figure 24 Binding affinity of Transferrin-PEG-AD to transferrin receptors on PC3 cells

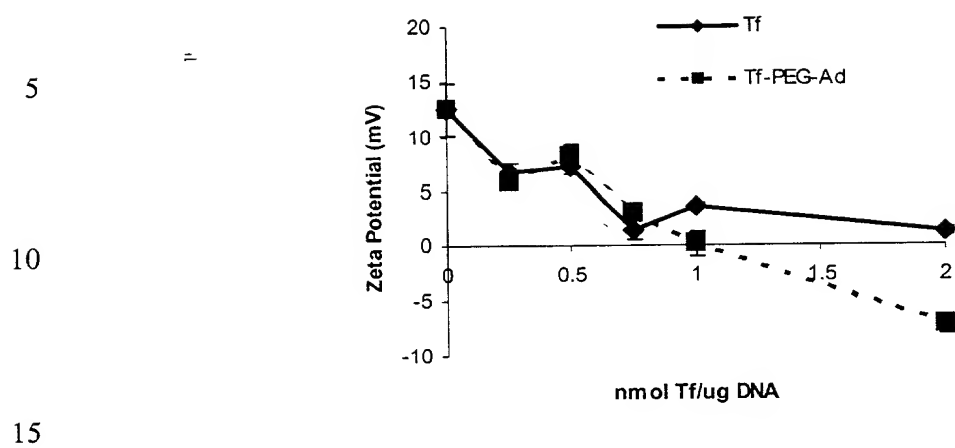


Figure 25 Zeta potential variation and particle size as a function of particle modification in transferrin and PEG-modified polyplexes

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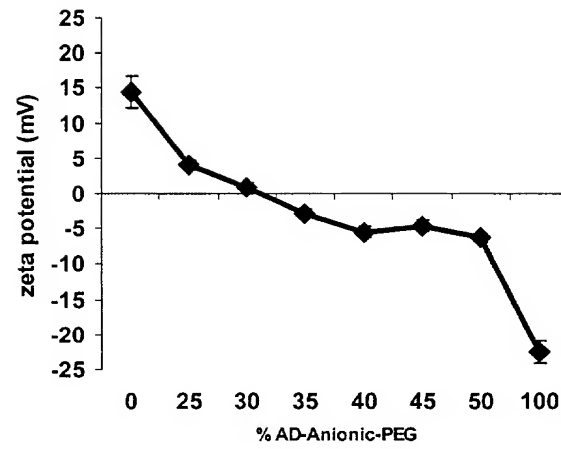


Figure 26 Zeta potential measurements, Ad-anionic-PEG

Stability in 150mM PBS using Ad-PEG  
1mg/ml DNA, 3+/- CDP

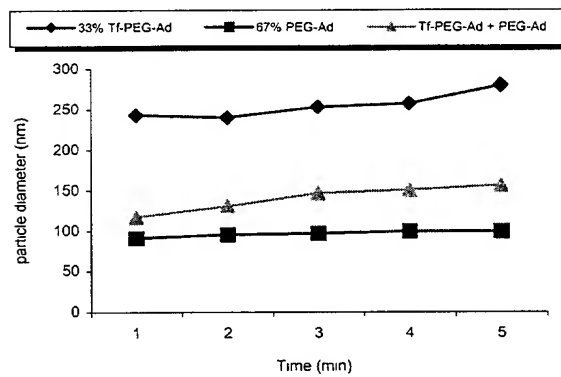


Figure 27

**Addition of increasing Tf-Modifier (balance is AD-PEG)  
1 mg/mL DNA, 3+/- CDP**

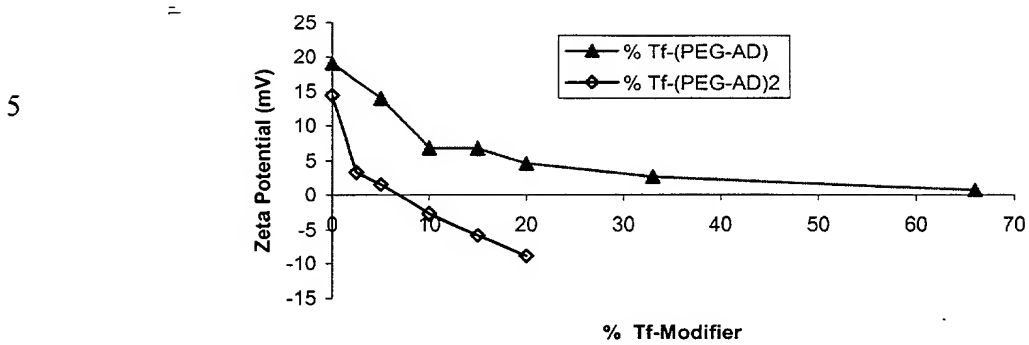
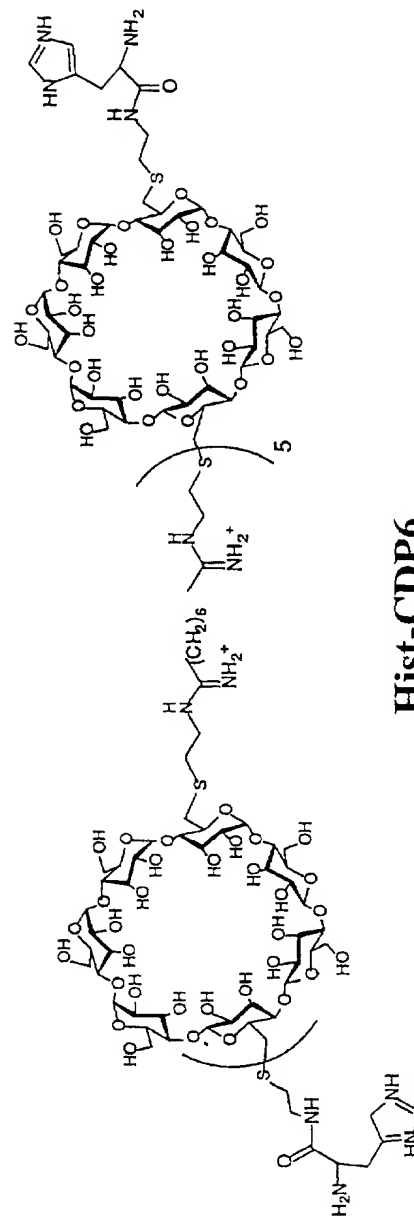
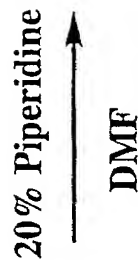


Figure 28

# Synthesis of Histidylated $\beta$ CDP6



**Hist-CDP6**

Figure 29

# pH-sensitive Polymers for Endosomal Escape

## Synthesis of secondary amine containing polymers

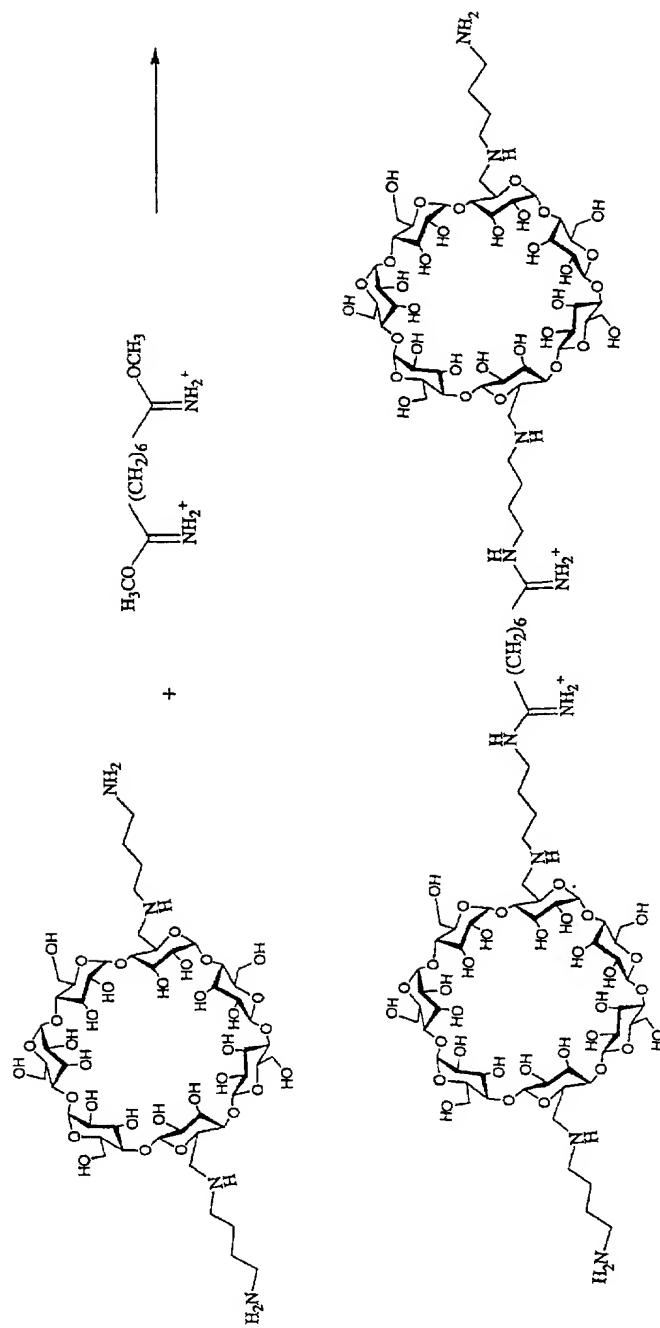


Figure 30